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Non-steroidal anti-psoriatic prodrugs. Hydrolysis and aminolysis of naphthyl esters in aqueous solution

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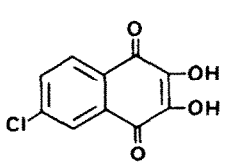
Summary

The anti-psoriatic compound, 6-chloro-2,3-dimethoxy-1,4-naphthalene diol diacetate (lonapalene) (**1**), undergoes ester hydrolysis and oxidation to give 2,3-dimethoxy-6-chloro-1,4-naphthoquinone (**3**). The hydrolysis reaction of **1** obeys the equation $k_{obs} = k_{H^+}[H^+] + k_0 + k_{HO^-}[HO^-]$ where k_{H^+}, k_0 and k_{HO^-} at 25°C are 1.42×10^{-5} $M^{-1} \cdot s^{-1}$, 3.68×10^{-8} s^{-1} and 1.78 $M^{-1} \cdot s^{-1}$, respectively. Both general acid and general base catalysis of **1** were observed, as well as an aminolysis reaction with primary amines. It is concluded that naphthyl esters undergo specific base catalysis by rate-determining hydroxide ion attack on the ester group to form a tetrahedral intermediate, whereas nucleophilic catalysis by amines proceeds by rate-determining proton transfer in the tetrahedral intermediate to base. It was also demonstrated that the hydrolysis rates of 1-naphthyl esters do not change greatly by altering the substituent in the 6- and 7-positions, and that the maximum shelf-life of **1** in aqueous formulations is approximately 1 month at room temperature and pH 4.

Introduction

Psoriasis is a chronic, genetically-determined disease characterized by inflammation of the skin and is estimated to afflict upwards of 2–6% of the worldwide population (Wheldon, 1976). The etiology of the disease is unknown and, unfortunately, no cure is available. Although many forms of treatment are currently being used, some of them more than 50 years old, these treatments provide only temporary relief from the symptoms of the disease. Such treatments include coal tars (with and without UV radiation), mercury compounds, psoralen and UV-A radiation, and retinoids (Baden, 1984). Among widespread use are treatments employing topical administration of anthralin and corticosteroids. Anthralin, however, is an irritating mutagenic compound, and the long-term side-effects of corticosteroids are well documented (Bickers, 1984).

In an attempt to overcome the severe limitations of previous treatments, we began a screening program several years ago to identify new non-steroidal anti-psoriatic agents (Simpson, 1984; Young, 1986). Early work led to the discovery of 6-chloro-isonaphthazarin (shown below), a com-



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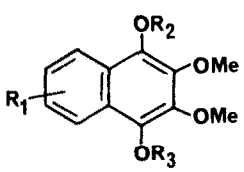
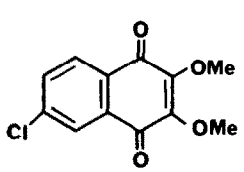
pound that demonstrated excellent anti-psoriatic activity after topical administration to man (Dumas, 1972; Jones, 1980, 1981). This compound, however, caused moderate sensitization and irritation in some patients and, from a pharmaceutical standpoint, suffered from an extreme propensity to undergo oxidation (Young, 1986). In an attempt to maintain pharmacological activity, eliminate the sensitization/irritation reaction, and improve the chemical stability, a series of analogs and prodrugs of 6-chloro-isonaphthazarin were synthesized and screened for activity. From this second generation of compounds, 6-chloro-2,3-dimethoxy-1,4-naphthalene diol diacetate (**1**) (trade name: lonapalene) has shown great promise as an anti-psoriatic agent (Young, 1986; Jones, 1984) and is now undergoing clinical trials in man (Lassus, 1985).

During the drug development program for lonapalene, we studied the solution chemistry of **1** and other naphthyl esters, and the results are reported herein. Compound **1** undergoes rapid hydrolysis to a pair of isomeric monoesters (**2**) which are further hydrolyzed and then oxidized to give the naphthoquinone analog (**3**). Since both **2** and **3** show good



1 $R_1 = Cl$; $R_2 = R_2 = COCH_3$
2 $R_1 = Cl$; $R_2 = COCH_3$; $R_3 = H$
5 $R_1 = CN$; $R_2 = R_3 = COCH_3$
6 $R_1 = CN$; $R_2 = COCH_3$; $R_3 = H$



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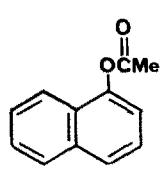

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activity against psoriasis (Young, 1986), it is most probable that **1** is a prodrug of the, as of yet, unidentified pharmacologically active agent. This paper reports our investigation on the aqueous hydrolysis of **1**, **2**, **5** and **6**, and on the aminolysis of **1** and **2**. Data on the aqueous hydrolysis and aminolysis of α-naphthylacetate (**4**) (Hawkins, 1955; Bruice, 1967) is included for comparison in the mechanistic studies.

Herein, we report that **1** and **2** are more stable than 6-chloro-isonaphthazarin, and that the stability of these naphthyl esters is still insufficient for formulation of the drug in an aqueous vehicle. Additionally, our study suggests that **1** and **2** undergo nucleophilic catalysis by amines. These latter results show that naphthyl ester substrates may be susceptible to enzyme-catalyzed hydrolysis inasmuch as the in vivo enzyme reaction is thought to be nucleophilic in nature (Makinen, 1976).

Experimental

Materials

Compounds **1**, **2**, **3** and **5** were prepared in the Institute of Organic Chemistry at Syntex (Palo Alto, CA) and the synthetic details of these and other anti-psoriatic compounds are described elsewhere (Jones, 1986). Radiolabeled 1,2,3,4,4a,8a-^{14}C-**1** was prepared in > 98% radiochemical purity (Parnes, 1986). *n*-Propylacetamide was prepared by reaction of acetic anhydride and *n*-propylamine in tetrahydrofuran. 4-Chlorophthalic acid, *n*-propylamine, tris-(hydroxymethyl)aminomethane (Tris), KH_2PO_4, H_3PO_4, D_2O, DCl and NaCl were of the highest grade commercially available (Aldrich or Mallickrodt) and were used without further purification. Mobile phases were prepared from HPLC-grade acetonitrile and distilled deionized water, or HPLC-grade hexane and methanol (Burdick and Jackson).

Apparatus

The separation and kinetic analysis of **1**, **2** and **3** was carried out using an HPLC system consisting of a Micromeritics Model 725 autoinjector, Model 110A Altex pump, Model 770 Spectra Physics spectrophotometric detector and an SP 4000 computing integrator. The following re-

versed-phase (RP) HPLC conditions provided a linear response throughout the range of 0.04–40 μg injected: column, Alltech Spherisorb S5-C6 (15 × 0.4 cm, 5 μm); mobile phase, acetonitrile–0.01 M potassium dihydrogen phosphate (adjusted to pH ~ 4 with phosphoric acid) (45 : 55, v/v); flow rate, 1 ml/min; detection, 242 nm; typical retention times, **3**, 7 min; **2**, 8 min; **1**, 12 min. The other naphthyl esters were also analyzed similarly. The separation and chromatographic identification of 4-chlorophthalic acid was done using the above conditions except that acetonitrile was omitted from the mobile phase (retention time = 12 min). *n*-Propylacetamide was separated from the hydrolysis products using the above conditions and a mobile phase consisting of 5% (v/v) acetonitrile in water (retention time = 13 min). A normal phase-HPLC method (NP-HPLC) was required to separate the 6- and 7-chloro isomers of **2**. The conditions used were: column, Altex Ultrasphere-Si (25 × 0.46 cm, 5 μm); mobile phase, hexane–methanol (96 : 4, v/v); flow rate, 1 ml/min; retention times, 7-chloro isomer, 20 min; 6-chloro isomer, 23 min. Collection and assay of radioactive HPLC fractions was carried out using an LKB Multi-Rac fraction collector and a Beckman LS 860 liquid scintillation counter. All sample activities were corrected for background counts and traces of radiolabeled impurities (~ 2%) in ^{14}C-labeled **1**. pHs were determined prior to the reaction using a Radiometer PHM 64 pH meter and Model GK2401C combination electrode. Electron impact mass spectra were obtained using a Varian MAT 1125 or 311A direct inlet mass spectrometer. NMR spectra were determined using a Bruker WM 300 FT-NMR spectrometer.

Kinetics

In order to obtain pseudo-first-order kinetics, the buffer concentration (~ 0.005 → 0.15 M) was always maintained in excess over the ester concentration (~ 1.5×10^{-5} M). In all experiments, buffer solutions were prepared shortly before use and the pH of each serial dilution was determined at the reaction temperature. Ionic strength was maintained constant at 0.15 M by the addition of sodium chloride. Stock solutions of esters **1**, **2** and **5** were prepared in acetonitrile and stored in the dark at 4°C when not in use. For the slower kinetic runs or those carried out at elevated temperatures, 100 ml of reaction solution and a small amount (~ 0.1–0.5 ml) of the ester stock solution were mixed well before 5 ml aliquots of the mixture were transferred to pretreated (with HCl and $(NH_4)_2SO_4$ to remove traces of base) clear ampules, flame sealed, and temperature equilibrated. At known time intervals, ampules were either removed from the temperature bath and refrigerated, or were assayed immediately by HPLC against a freshly-prepared reference solution of **1**. Upon removal of the last sample, all of the stored samples were analyzed on the same day. Faster reaction rates were obtained by removing aliquots from a single reaction vessel at given time intervals and immediately quenching with acetate buffer to a final pH of ~ 4–5, and then assaying as before. In a typical experiment, 8–12 samples were analyzed, and the peak area integrations were converted to concentrations or % remaining values by use of linear response calibration curves determined earlier for **1**, **2** and **3**.

pK_a determinations

The pK_a values for the 6- and 7-chloro isomers of **2** and a 50 : 50 mixture of these isomers were determined potentiometrically by the method of Albert and Sarjeant (Albert, 1971) at λ = 242 nm using an HP-8450A spectrophotometer. In a typical determination 0.001 M potassium dihydrogen phosphate was used as a buffer during the addition of small amounts of concentrated sodium hydroxide to the temperature-equilibrated solution.

Product identification

UV spectra of the major RP-HPLC peaks were obtained by monitoring the HPLC effluent using an HP-8450A spectrophotometer equipped with an 8 μl flow cell. Large scale separation and collection of the major peaks using acetonitrile–water (45 : 55 v/v) as the HPLC mobile phase afforded the two isomers of **2**, and **3**. These were confirmed by their electron impact mass spectra and ^{1}H-NMR spectra. Separation of the isomers of **2** was carried out by NP-HPLC and positive identification was made by the NMR Overhauser effect (Jones, 1986) and by comparison with

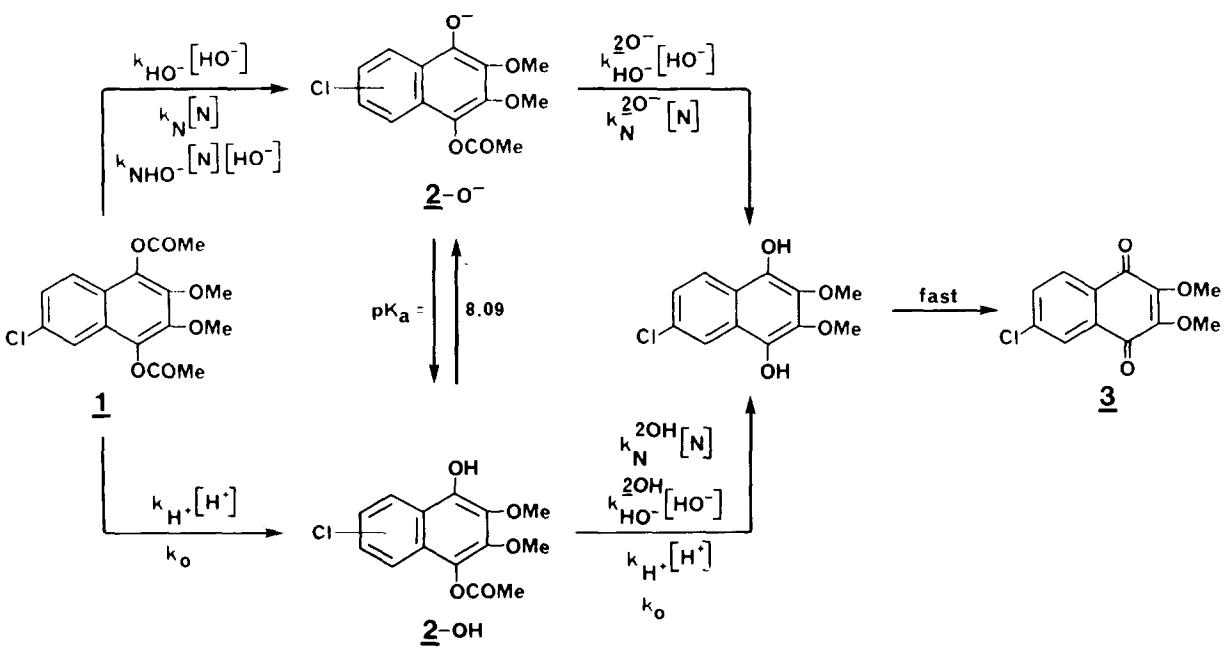
authentic samples. Identification of **3** was verified by comparison of HPLC retention times with an authentic sample. In most cases, both spectral (UV, NMR and MS) and chromatographic (RP- and NP-HPLC) comparisons were made. Identification of *n*-propylacetamide, the aminolysis product of **1** with *n*-propylamine, was carried out by showing identical RP-HPLC retention times and UV spectra with that of an authentic sample. The identities of the radiolabeled peaks in the HPLC chromatograms of ^{14}C-labeled **1** and its degradation products were determined by chromatographic retention time only.

Results and Discussion

In this, our first of 3 papers on the chemistry of new non-steroidal anti-psoriatic agents (Powell, 1986), we report the effect of solution pH, reaction temperature and various buffers on the degradation of **1** and **2**. We are interested in such in vitro studies because: (i) they provide a model system for delineating factors governing the shelf-life of **1** in the water-based cream formulations under development; (ii) the degradation product **2** also demonstrates remarkable anti-psoriatic activity in man and so its formation rate from **1** is germane; and (iii) the degradation of **1** and **2** by buffer nucleophiles may yield information relating to the mechanism of in vivo enzyme-catalyzed hydrolysis of ester substrates — a reaction thought to proceed via a nucleophilic mechanism (Makinen, 1976).

Degradation products of 1 and 2

The degradation pathways for **1** are shown in Scheme 1. In Scheme 1 and what follows, the rate constant subscripts denote the catalytic species and the superscripts denote the protonation state of the substrate. For example, $k_{HO^-}[HO^-]$, k_N^{2OH} and $k_{NHO^-}[N][HO^-]$ are the rate contributions by specific hydroxide ion catalysis of **1**, unassisted nucleophilic attack by an amine on the species **2OH**, and hydroxide ion catalysis of nucleophilic attack by an amine, respectively. Under all reaction conditions employed, hydrolysis of the diester prodrug, **1**, gave initially an approximate 50 : 50 mixture of the 6- and 7-chloro substituted monoesters (designated collectively as **2**). For the



Scheme 1

purposes of this study, the 6- and 7-chloro isomers of **2** could be treated collectively, inasmuch as partial hydrolysis of **1** afforded equal amounts of each isomer, and because the hydrolysis rates of the 6- and 7-isomers of **2** were nearly identical. The pK_as for the 6- and 7-isomers of **2** were also determined herein to be nearly identical at 8.05 ± 0.01 and 8.12 ± 0.03, respectively. Each of these monoesters existed in the protonated (**2**OH) and deprotonated ($\mathbf{2O}^-$) forms, depending on the pH of the reaction solution. Subsequent hydrolysis of **2** followed by rapid oxidation afforded primarily 2,3-dimethoxy-6-chloro-1,4-naphthaquinone (**3**). No additional HPLC peaks were detected which might correspond to the hydroquinone during any of the experiments. In the presence of propylamine buffers, the reaction of **1** proceeded almost exclusively by aminolysis as shown by the quantitative formation of *n*-propylacetamide.

To prove that all hydrolysis products of **1** were detected and accounted for, the hydrolysis of ^{14}C-labeled **1** was carried out in the acidic, neutral and basic pH regions. The reported percent-remaining values of Table 1 show that 100% mass balance was obtained to at least one half-life when fractions coincident to **1**, **2**, **3** and the solvent front were collected and counted. The primary degradation product of radiolabeled **1** was identified as **2** which underwent subsequent hydrolysis and oxidation to give **3**. The small amount (~ 1%) of radioactivity found in the solvent front proved to be derived from several compounds. When the retention times were lengthened using a 100% aqueous mobile phase, one of these compounds was identified as 4-chlorophthalic acid. These minor degradation products (< 1% at t_{90}) have not been included in Scheme 1 or the following kinetic analysis because of their negligible contribution to the yield of degradation products. First-order rate constants determined from the loss of radiolabeled **1** were in close agreement with rate constants determined by a UV-spectrophotometric assay method (vide infra).

TABLE 1

Product distribution and mass balance for hydrolysis of ^{14}C-labeled-1 to approximately 90% remaining at 25°C [a]

pH	% Remaining				Mass balance
	1	2	3	c	
1.0 [b]	88.21	5.89	3.47	2.10	99.67
4.0	91.56	4.68	2.63	0.81	99.68
9.2	87.30	11.79	0	0.95	100.0

[a] The reported % remaining values have been corrected for approximately 2% of radiolabeled impurities in the stock solution of **1**.

[b] 0.1 M HCl, 40°C.

[c] Undefined peaks eluted at the solvent front.

Degradation kinetics

Hydrolysis of 1

In all experiments, the disappearance of **1** followed pseudo-first-order kinetics (Fig. 1). The hydrolysis rates of **1** were determined at several temperatures (25, 60 and 80°C) and pHs (pH 0–12) using sodium chloride to maintain constant ionic strength at 0.15 M (Fig. 2). At pHs less than 2, rate constants were determined in dilute hydrochloric acid or deuterated (> 98% isotopic purity) hydrochloric acid solutions. From pH 3 to 6, the rate constants were obtained from solutions buffered with acetate at 0.025 M, or by extrapolation of serially-diluted buffer to zero buffer concentration. Rate constants determined by either of

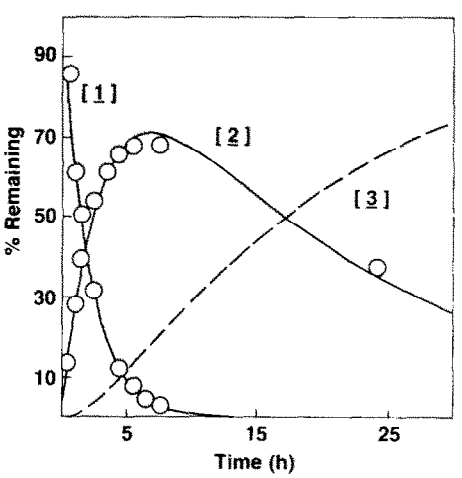


Fig. 1. Reaction of **1** at pH 9.27 ([Tris] = 0.15) and 25°C to give **2** and **3**. The best fit line for **2** was obtained by non-linear least-squares analysis of Eqn. 3. The dashed line is the calculated yield of naphthoquinone **3** and is equal to $100 - [\mathbf{1}]_t - [\mathbf{2}]_t$.

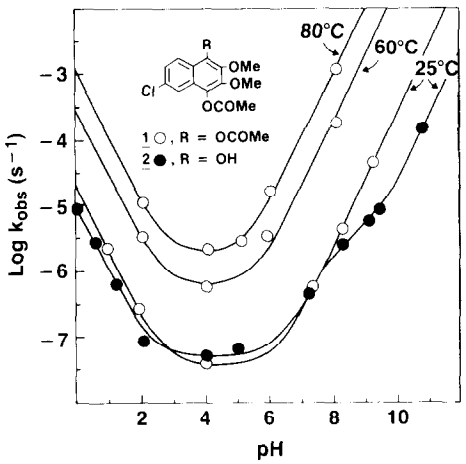


Fig. 2. Log(k_{obs})–pH profiles for the reaction of **1** and **2**. The best fit rate constants obtained from nonlinear least-squares analysis of Eqns. 1 and 2 are used to generate the profiles shown.

these methods in this pH region were similar, indicating only slight buffer catalysis. At pHs greater than 6, however, the extrapolation method had to be used because of a significant contribution to the overall rate by the basic buffer component, especially when amine buffers were used. In general, plots of k_{obs} versus buffer base concentration were linear over the buffer range of ~ 0.015–0.15 M.

In the absence of buffer catalysis, a plot of log k_{obs} versus pH for **1** (Fig. 2) showed 3 distinct regions of curvature having a slope of −1 in the acid region, +1 in the base region and a short plateau near pH 4 approaching a slope of 0, in accord with Eqn. 1:

$$k_{obs} = k_{H^+}[H^+] + k_0 + k_{HO^-}[HO^-] \qquad (1)$$

The effects of hydronium ion ($k_{H^+}[H^+]$) and hydroxide ion ($k_{HO^-}[HO^-]$) are minimized about pH 4; at this pH the major rate contribution is due to catalysis by water (k_0). The secondary rate constants derived from non-linear least-squares analysis of the data shown in Fig. 2 are given in Table 2.

Inspection of Fig. 2 shows this behavior is observed over a range of temperatures. The temperature-dependent hydrolysis rates of **1** show linear Arrhenius behavior (Fig. 3) and give the following values of $\Delta H^{\ddagger}$ (kcal · mol^{-1}) and $\Delta S^{\ddagger}$ (cal · $mol^{-1} \cdot K^{-1}$): k_{H^+} ($M^{-1} \cdot s^{-1}$), 13.3, −35.9; k_0 (s^{-1}), 14.5, −43.6; k_{HO^-} ($M^{-1} \cdot s^{-1}$), 9.9, −23.8, respectively. It becomes readily apparent that formulations containing significant amounts of water are unacceptable because the t_{90} for **1** in aqueous solution (pH 4) at 25°C is approximately 1 month.

Metal ions such as Fe^{2+} and Cu^{2+} did not catalyze the hydrolysis of **1**, as expected for reaction of an ester unable to coordinate such divalent cations (Fife, 1982; Guenzet, 1972). In support of this finding, added EDTA had no effect on the reaction rate. Experiments carried out aerobically and anaerobically also showed identical reaction rates, indicating that oxidative degradation of **1** (and **2**) is negligible, even at the pH of maximum stability.

Hydrolysis of 2

Degradation of **2** in aqueous solution also followed pseudo-first-order kinetics. In the hydrolysis of **2**, however, a deviation from Eqn. 1 at pH ~ 8 was observed due to the slower reaction of $2O^-$. Incorporation of a pK_a term into Eqn. 1 to account for hydroxide ion catalysis of the proto-

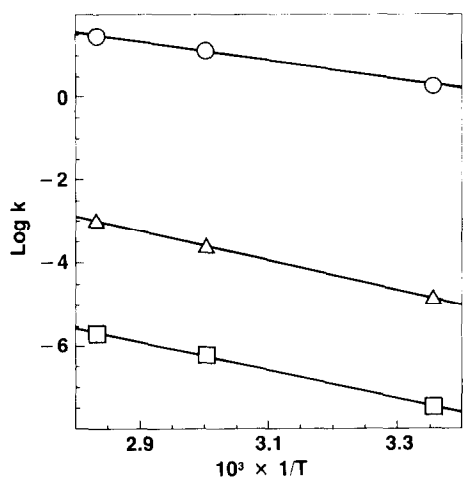


Fig. 3. Arrhenius plots for the hydronium ion (△), water (□) and hydroxide ion (○) catalyzed hydrolysis of **1**. The values of E_a (kcal · mol^{-1}) and log A for k_{H^+}, k_0 and k_{HO^-} are 13.9, 5.4; 15.2, 3.7; and 10.6, 8.0, respectively.

TABLE 2

Summary of leaving group pK_as and rate constants for the hydrolysis and aminolysis of 1 and 2 and related naphthyl esters

Leaving Group			k_{H^+} ($M^{-1} \cdot s^{-1}$) [a]	k_0 (s^{-1})	k_{HO^-} ($M^{-1} \cdot s^{-1}$) [a]	k_N ($M^{-1} \cdot s^{-1}$) [c]	k_N ($M^{-1} \cdot s^{-1}$) [d]
Drug	pK_a	Temp (°C)					
1	8.09	25	$(1.42 \pm 0.07) \times 10^{-5}$ [b]	$(3.68 \pm 0.26) \times 10^{-8}$	1.78 ± 0.07	3.6×10^{-4}	4.70
		60	$(2.16 \pm 1.22) \times 10^{-4}$	$(6.25 \pm 2.90) \times 10^{-7}$	12.9 ± 4.7	i	i
		80	$(8.32 \pm 1.50) \times 10^{-4}$	$(1.92 \pm 0.21) \times 10^{-6}$	28.1 ± 3.2	i	i
2OH	8.51 [e]	25	$(7.19 \pm 0.77) \times 10^{-6}$	$(4.95 \pm 0.67) \times 10^{-8}$	2.12 ± 0.26	2.8×10^{-5}	i
2O⁻	10.10 [e]	25	f	f	0.138 ± 0.022	3.1×10^{-5}	5.5×10^{-3}
4	9.30 [g]	25	f	f	1.40 [h]	i	0.175 [h]
5	7.20	25	i	i	4.09	i	i
6O⁻	9.65 [e]	25	i	i	0.264	i	i

[a] These rate constants have been corrected for activity using the following temperature-dependent activity coefficients of f_{H^+} and f_{HO^-}: 25°C, 0.78 and 0.77; 60°C, 0.76 and 0.75; 80°C, 0.75 and 0.74, respectively (Bates, 1973).

[b] The bimolecular rate constant for the degradation of **1** in deuterated hydrochloric acid at 25°C is $(1.37 \pm 0.06) \times 10^{-4}$ $M^{-1} \cdot s^{-1}$.

[c] k_N for reaction with tris-(trihydroxymethyl)aminomethane.

[d] k_N for reaction with *n*-propylamine.

[e] Determined from the best fit line of $\Sigma\sigma = (-4.330 \pm 0.012)pK_a + (4.319 \pm 0.109)$ where $\Sigma\sigma$ is for phenols and naphthols (Perrin, 1980).

[f] The contributions made to the overall rate by the reaction of **2O⁻** with either H^+ or H_2O are expected to be negligible because, in the pH region where these terms dominate (pH 0 → 6), the major fraction of **2** is present in the protonated form, **2OH**.

[g] Fasman (1976).

[h] Reaction of 1-naphthyl acetate in *n*-butylamine buffers at 30°C ($\mu = 1.0$) (Bruice, 1967).

[i] Not determined.

nated and deprotonated forms of **2** affords the following expression:

$$k_{obs} = \frac{k_{H^+}[H^+] + k_0 + k_{HO^-}^{2OH}[HO^-]}{([H^+] + K_a)/[H^+]} + \frac{k_{HO^-}^{2O^-}[HO^-]}{(K_a + [H^+])/K_a} \quad (2)$$

where K_a is the acid dissociation constant for **2** ($K_a = 8.13 \times 10^{-9}$ at 25°C) and $k_{HO^-}^{2OH}$ and $k_{HO^-}^{2O^-}$ are the rate constants for specific hydroxide ion catalysis for the protonated and deprotonated forms of **2**, respectively. Non-linear least-squares analysis of log k_{obs} versus pH (Fig. 2) using Eqn. 3 gives the secondary rate constants shown in Table 2.

The complimentary formation and subsequent disappearance of **2** from **1** could also be fit accurately using Eqn. 3 derived from an A → B → C reaction scheme where the observed rate constants k_1 and k_2 correspond to reactions A → B (**1** → **2**) and B → C (**2** → **3**), respectively.

$$\text{percent } \mathbf{2} \text{ remaining} = \frac{100\, k_1}{(k_2 - k_1)} \times \{e^{-k_1 t} - e^{-k_2 t}\} \quad (3)$$

The concentration of **2** initially increases (where the rate of formation of **2** equals the rate of drug loss of **1**) before it drops (due to reaction of **2** itself), as shown in Fig. 1.

General catalysis of 1 by acetate buffer

Defining the extent of general acid and general base (or nucleophilic) catalysis was done by studying the degradation of **1** in acetate buffers at several pHs (Fig. 4). These kinetics were carried out at 80°C to accelerate an otherwise inordinantly slow reaction. The observed rate constants obeyed the general expression of Eqn. 4 where

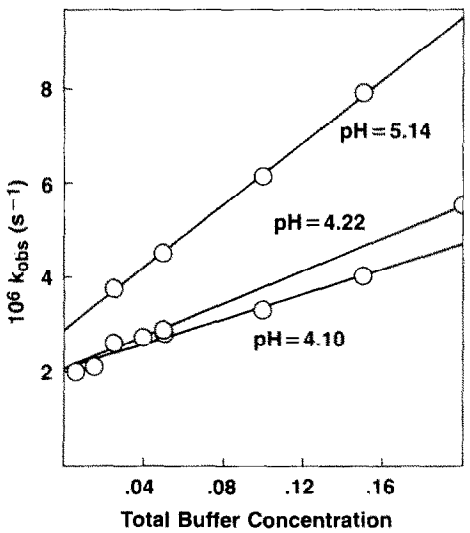


Fig. 4. Dependence of k_{obs} for reaction of **1** at 80°C on the total acetate buffer concentration B_T at various pHs. The slopes are denoted k′ and the intercepts are equal to $k_{H^+}[H^+] + k_0$.

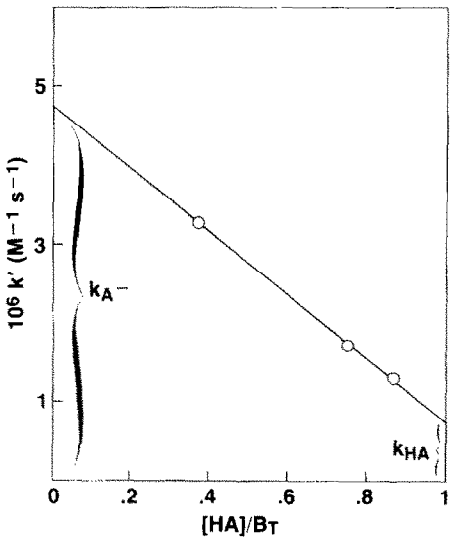


Fig. 5. Dependence of the apparent rate constant for buffer catalysis k′ on the composition of acetate buffer for reaction of **1** at 80°C. The 0 and 1 intercepts are k_{A^-} and k_{HA}, respectively.

k_{HA} and k_{A^-} are the rate constants denoting catalysis

$$k_{obs} = k_{H^+}[H^+] + k_0 + k_{HA}[HA] + k_{A^-}[A^-] \quad (4)$$

by acetic acid and acetate, respectively. Rearranging and dividing both sides by the total buffer concentration B_T, gives Eqn. 5:

$$k' = \frac{k_{obs} - k_{H^+}[H^+] - k_0}{B_T}$$

$$= k_{HA}[HA]/B_T + k_{A^-}\{(B_T - [HA])/B_T\} \quad (5)$$

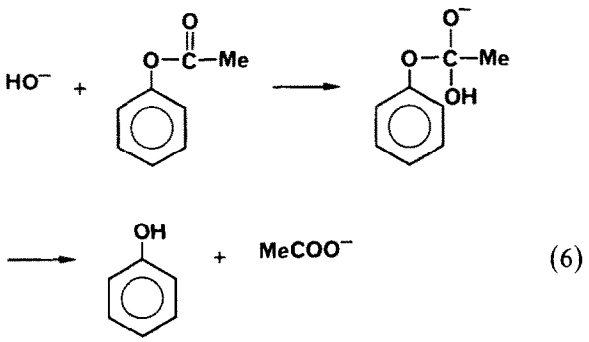
Thus, a plot of the pseudo-first-order rate constants, k_{obs}, versus B_T at a constant pH affords $k_{H^+}[H^+] + k_0$ as the intercept and k′ as the slope (Fig. 4). A secondary plot of the apparent rate constants k′ versus the fraction of free acid present, $[HA]/B_T$, gives k_{A^-} and k_{HA} as the 0 and 1 intercepts, respectively. Such a plot for the reaction of **1** in acetate buffers at 80°C is shown in Fig. 5; the derived values of k_{HA} and k_{A^-} are 7.37×10^{-6} $M^{-1} \cdot s^{-1}$ and 4.74×10^{-5} $M^{-1} \cdot s^{-1}$, respectively. Although acetic acid is a fairly strong acid and acetate is a relatively weak base, the rate constant for catalysis by acetate is ~ 6-fold greater than that for acetic acid indicating that general base or nucleophilic catalysis is the dominant buffer reaction at this pH. Previous studies of the acetate-catalyzed hydrolysis of substituted phenyl acetates have shown conclusively that both nucleophilic and general base catalysis are operative and that the fraction of each depends on the pK_a of the leaving group (Gold, 1968). For phenyl acetates with good leaving groups having pK_as near or less than the pK_a of acetate, direct nucleophilic attack is the predominant reaction; otherwise acetate exhibits general base catalysis. Since **1** has a leaving group pK_a greater than the pK_a for acetate, the acetate-catalyzed degradation of **1** probably follows a general base-catalyzed mechanism.

The contribution to the overall degradation rate by hydronium ion ($k_{H^+}[H^+]$) is important in the highly acidic region (pH < 3). Suffice it to say that the reaction rates, activation parameters and the solvent isotope effect for specific acid catalysis of **1** at 25°C ($k_{H^+}/k_{D^+} = 1.08 \pm 0.05$) are of the expected magnitudes for an A_{AC^2} mechanism of

acid-catalyzed ester hydrolysis (Yates, 1967; Said, 1981). The base-catalyzed hydrolysis reaction predominates throughout most of the pH range (pH > 5) and is the subject of the next section.

Mechanism of base-catalyzed naphthyl ester hydrolysis

The shelf-life of **1** is ~ 1 month at pH ~ 4, and even less in neutral pH solution where specific base catalysis predominates. For most esters, the rate of base-catalyzed hydrolysis is dependent on the rate-determining step of the hydrolysis reaction; i.e. whether formation of the tetrahedral intermediate by nucleophilic attack (first step of Eqn. 6), or breakdown of this intermediate (second step) is rate-determining. For both of these mechanisms, the hydrolysis rate is reflected in the pK_a of the leaving group, albeit slightly differently.



(6)

Correlation in Fig. 6 of the hydroxide-catalyzed hydrolysis rates at 25°C of alkyl, phenyl (Bruice, 1960; Kirsch, 1964) and naphthyl esters with the pK_a of the leaving group shows that the phenyl and naphthyl esters exhibit the slowest hydrolysis rates at constant leaving group pK_a, and that the rates of naphthyl ester hydrolysis are only mildly dependent on the leaving group ability. It is well established that the rate-determining step for alkyl ester hydrolysis is breakdown of the tetrahedral intermediate (Eqn. 6), largely due to the poor leaving ability of alkyl alcohols, and this is reflected in the steeper slope of the log k_{HO^-} versus leaving group pK_a plot (Fig. 6). On the other hand, the phenolate ion is a good leaving group, and it has been proposed that the rate-determining step for phenyl ester hydrolysis is hydroxide attack to form the tetrahedral intermediate (Bruice, 1960; Kirsch, 1964; Cohen, 1973). Inspection of Fig. 6 shows that naphthyl esters are less sensitive than phenyl esters to the nature of the leaving group. This is expected for a reaction involving rate-determining nucleophilic attack on a sterically-hindered substrate with a good leaving group. Naphthyl acetate (**4**) is included in this series of 2,3-methoxy-substituted compounds because the *o*-methoxy group is not expected to cause an appreciable rate retardation due to steric hindrance. This is supported by the similar rates of base-catalyzed hydrolysis of phenyl acetate and *o*-methoxyphenyl acetate (Nishioka, 1975). This low sensitivity of rate constant upon leaving group pK_a is well illustrated by comparing the reactivity of α-naphthyl acetate (**4**) and 1,4-diacetoxy-6-cyano-2,3-dimethoxy naphthalene (**5**). Although the leaving group pK_a for **4** (Fasman, 1976) is more than two pK_a units higher than that for **5** (Table 2), the k_{HO^-} rate constant for **5** is only 3 times larger than k_{HO^-} for reaction of **4** (Table 2).

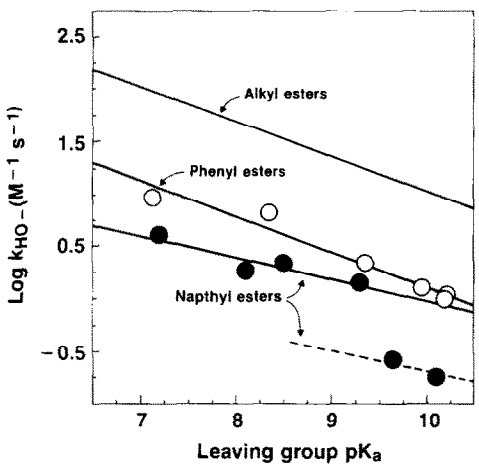


Fig. 6. Correlation of log k_{HO^-} ($M^{-1} \cdot s^{-1}$) with the pK_a of the leaving group. The points for the alkyl esters lie outside the range shown and therefore are not plotted. The dashed line for **2O⁻** and **6O⁻** is drawn parallel to the line for the neutral naphthyl esters for comparison purposes only.

The k_{HO^-} values for the two anionic esters (1-acetoxy-6-chloro-2,3-dimethoxynaphthalene-4-oxide, **2O⁻**, and 1-acetoxy-6-cyano-2,3-di-

methoxynaphthalene-4-oxide, $\mathbf{6O^-}$) fall approximately 0.75 log units below the line for the neutral naphthyl acetates (Fig. 6). This rate retardation for the negatively-charged esters could be due to an electrostatic repulsion effect between the reactants (ArO^- and HO^-), or it could be due to a change in the rate-determining step; i.e. to the breakdown of the tetrahedral intermediate because of the poor leaving group ability of the ArO^{2-} dianion. The first explanation is preferred because phenyl esters of similar leaving group pK_a (9–10) as ArO^- show rate-determining nucleophilic attack by HO^-, and because a 5-fold rate reduction (the difference between the lines for charged and uncharged naphthyl acetates of Fig. 6) is not unexpected for an electrostatic repulsion effect in a reaction of this type (Dahlberg, 1983).

Nucleophilic catalysis of 1 by amines

The aminolysis rates for the reaction of **1** and **2** were obtained from experiments carried out in amine-buffered media using tris-(hydroxymethyl) aminomethane (pK_a 8.08) (Bates, 1961) and *n*-propylamine (pK_a 10.64) (Fasman, 1976). In Tris buffers, the observed rate constants in the basic region were found to follow the general relationship:

$$k_{obs} = k_{HO^-}[HO^-] + k_N[N] + k_{NHO^-}[N][HO^-] \quad (7)$$

where [N] refers to the free (unprotonated) amine concentration. Use of a more complete expression to include complex general acid and general base terms was not necessary to fit the observed kinetics. Rate constants were determined by either monitoring the first-order disappearance of **1** or by following the appearance of **2**; in the latter case rate constants were derived from an $A \rightarrow B \rightarrow C$ fit of the data. Plots of k_{obs} versus [N] at a single pH gave $k_N + k_{NHO^-}[HO^-]$ as the slope and $k_{HO^-}[HO^-]$ as the intercept (Fig. 7). A secondary plot of these slopes versus $[HO^-]$ determined at each pH afforded k_{NHO^-} as the secondary slope and k_N as the secondary intercept. The rate constants k_N and k_{NHO^-} for reaction of **1** and Tris at 25°C are $3.6 \times 10^{-4}\ M^{-1} \cdot s^{-1}$ and $8.29\ M^{-1} \cdot s^{-1}$, respectively. For the reaction of **1** with *n*-propylamine, the contribution to the overall rate made by the $k_{NHO^-}[HO^-]$ term was found to be negligible and the rate expression of Eqn. 7 was simplified to give Eqn. 8. In this case, a plot of k_{obs} versus [N] gives $k_{HO^-}[HO^-]$ as the intercept and k_N as the slope directly.

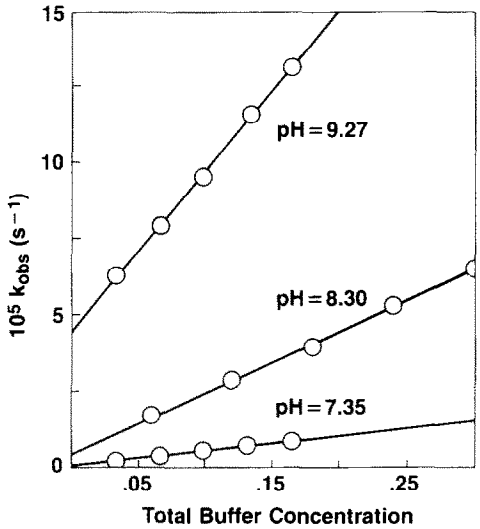


Fig. 7. Effect of Tris buffer concentration on the rate constant for degradation of **1** (25°C) at several pHs.

$$k_{obs} = k_{HO^-}[HO^-] + k_N[N] \quad (8)$$

Nucleophilic catalysis of 2 by amines

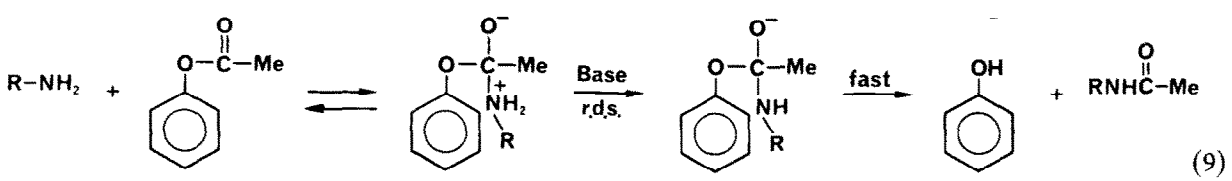
The analysis of rate constants for the aminolysis reaction of **2** with *n*-propylamine or Tris was not as straightforward as for reaction of **1**, in that the protonated (**2OH**) and deprotonated ($\mathbf{2O^-}$) forms of **2** may contribute to the rate differently. When Eqn. 8 is corrected for the fractions of protonated ($[H^+]/\{[H^+] + K_a\}$) and deprotonated ($K_a/\{K_a + [H^+]\}$) **2** in solution, the slope of a plot of k_{obs} versus [N] has four unknowns: k_N^{2OH}, $k_N^{2O^-}$, $k_{NHO^-}^{2OH}$ and $k_{NHO^-}^{2O^-}$. Although the terms comprising the intercept ($k_{HO^-}^{2OH}[HO^-] + k_{HO^-}^{2O^-}[HO^-]$) of such a plot are easily sorted out by construction of a $\log(k_{obs})$–pH profile (Fig. 2), it is immediately apparent that the slope of such a buffer plot is complex and that resolution of these terms cannot be achieved unless some approximations are made. Fortunately, the rate data pro-

vided for the similar reaction of **1** in Tris buffers shows that $k_{NHO^-}[HO^-] \ll k_N[N]$ under the experimental conditions used and thus the $k_{NHO^-}[HO^-]$ term can be neglected for **2** (e.g. as in Eqn. 8), especially if the basic buffer component is at low concentration. Moreover, the contribution to the overall rate by $k^{2O^-}_{NHO^-}$ should be even less than $k^{2OH}_{NHO^-}$ since electrostatic repulsion between the reactants ($2O^-$ and HO^-) should retard the reaction rate. Additionally, the k_{NHO^-} term(s) for reaction of **2** in *n*-propylamine buffers could not be resolved, as previously reported for the reaction of naphthyl acetate (**4**) with *n*-butylamine reported earlier (Hawkins, 1955; Bruice, 1967). Thus, the slope of a plot of k_{obs} versus [N] for reaction of **2** is approximated by $k^{2OH}_N[H^+]/\{[H^+] + K_a\} + k^{2O^-}_N K_a/\{K_a + [H^+]\}$ such that the k_N values for the reaction of **2OH** and $2O^-$ with Tris and propylamine (given in Table 2) are easily resolved by rate measurements at different pHs.

Mechanism of naphthyl ester aminolysis

The degradation of **1** and **2** is strongly amine buffer catalyzed, as shown by the reasonably large values of k_N in Table 2. In this study, amine catalysis has been shown to be nucleophilic in nature, and is derived from the attack of free base amine on the ester. The rates of amine attack on substrate **1** can be used to estimate the nucleophilic reactivity for other structurally-similar amines of comparable pK_a (because, for most amines, nucleophilicity roughly parallels amine base strength). Furthermore, the amine rate data reported herein support the mechanism proposed by Satterwait and Jencks for the aminolysis of aryl esters—namely pre-equilibrium formation of a tetrahedral intermediate followed by rate-determining proton transfer from the amino portion of the tetrahedral intermediate to base before rapid collapse of the deprotonated intermediate to give the products, i.e. as in Eqn. 9 (Satterwait, 1974).

The Bronsted β value derived from the slope of a plot of log k_N versus pK_a of the amine base for the reaction of **1** with Tris and propylamine is 1.5 (β^1_{nuc}) whereas reaction of $2O^-$ with the same amines yields a value of 1.1 ($\beta^{2O^-}_{nuc}$). (The value for β^{2OH}_{nuc} could not be obtained because the $k^{2OH^-}_N[N]$ term makes a negligible contribution to the overall rate in propylamine buffers.) Tris is known to exhibit greater steric hindrance than other primary amines resulting in a marked negative deviation of Tris from primary amine Bronsted plots for nucleophilic catalysis such that the β_{nuc} values, when compensated for steric hindrance of Tris, are probably somewhat smaller, perhaps 0.8–1.1. A β_{nuc} value near 1.0 means that the sensitivity of nucleophilic attack (of amine on ester) to substituents on the amine is quite similar to protonation of the amine, i.e. as if the attacking and catalyzing amine molecules in the transition state have a shared charge of approximately +1. This is expected for a rate-determining proton transfer reaction from the tetrahedral intermediate to base (Satterwait, 1974). Additional support for rate-determining proton transfer comes from the observation that $2O^-$ reacts with Tris slightly faster than **2OH**, in contradistinction to the observed ratio of rates for the hydroxide-catalyzed reaction ($k^{2O^-}_{HO^-}/k^{2OH}_{HO^-} = 0.065$). This may be due to favorable electrostatic catalysis between the naphthoxy group of $2O^-$ and the positively-charged nitrogen in the transition state, or possibly, by involvement of a cyclic transition state whereby the naphthoxy group catalyzes the proton transfer reaction through a molecule of water. Regardless of which of these mechanisms is operative, a slow proton transfer step from the tetrahedral intermediate to a base molecule does explain $k^{2O^-}_N > k^{2OH}_N$, whereas rate-determining nucleophilic attack or rate-determining breakdown of the deprotonated tetrahedral intermediate does not.



(9)

Acknowledgements

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